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Ectomycorrhizal succession in a Scottish birch wood

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INTRODUCTION

A succession of mycorrhizal fungi is known to occur on roots of birch (MASON *et al.*, 1982; FLEMING *et al.*, 1984; LAST *et al.*, 1984). DEACON, DONALDSON and LAST (1983) distinguished between those fungi that occurred early in the succession, such as species of *Laccaria* and *Hebeloma* and those that occurred later, such as *Lactarius pubescens* (Fr.: Krombh.) Fr. and *Leccinum* spp., according to their ability to colonize seedlings grown in a glasshouse in cores of soil collected from beneath their fruitbodies - 'early-stage' fungi readily colonized seedlings but 'late-stage' fungi did not do so. FLEMING (1984) later demonstrated that *L. pubescens* and *Leccinum* spp. could colonize the roots of seedlings planted in undisturbed positions around mature trees known to bear the mycorrhizas of these fungi. Infection could be reduced or prevented if the planting positions were first cored, to isolate inoculum from contact with a mature host. It thus appears that 'late-stage' fungi require a food base provided by a mature tree in order to have sufficient inoculum potential to colonize birch seedling roots growing in unsterilized substrates.

All the above experiments were done on a relatively young (<14 years old) stand of birches (*Betula pendula* Roth. and *B. pubescens* Ehrh.) growing on the Bush Estate, in what was formerly agricultural soil. The following experiments were done to see if the principles established through work on Bush Estate apply equally to other sites, in particular semi-natural birch woodland in the Scottish Highlands. Struan wood, Calvine, Perthshire (OS map reference NN7966; 56°45'N, 4°0'W) was selected as being typical of relict birch woodlands (GIMINGHAM, 1984), it consists exclusively of old scattered birch trees (mostly *B. pubescens*) with numerous stunted birch saplings in the heavily grazed ground cover. Soils vary over the wood and include brown earths (pH 5.2), acid brown earths (pH 4.0; A horizon) and deep accumulations of humus (pH 3.8) directly overlying parent material.

METHODS

Glasshouse bio-assay

Composite samples of soil, humus and plant litter were collected, with a trowel, in autumn of 1982 and 1984 from beneath the spatially separated fruitbodies of a range of mycorrhizal fungi (see Table 1), similar to those used by DEACON *et al.*, (1983) and typical of northern birchwoods (WATLING, 1984). Each soil sample was divided into two equal portions. One portion was washed over a sieve and the residue was assessed for mycorrhizas, identified where possible by morphology and using reference material at Edinburgh. The remaining portion was used to fill 5.0 cm square pots each of which was then planted with an aseptically germinated birch seedling. The number of replicate pots (≥ 3) varied with the volume of soil available. The pots were randomised and kept in a heated glasshouse (20 ± 8°C; 20 h photoperiod) for either 141 days (1982) or 110 days (1984) when the seedlings were harvested and assessed for mycorrhizas. Naturally regenerating seedlings (< 1 year old) also were collected in 1982 from one site; they were assessed for mycorrhizas and the soil from around the seedlings was assayed as above.

Field outplanting

Three sites were selected in autumn 1983 within Struan wood: one where *Amanita muscaria* (Pers.:Fr.) Hooker had produced fruitbodies (site 1) another where *Leccinum roseofracta* Watl. and *Laccaria laccata* (Scop.:Fr.) Berk. + Br. fruitbodies had been observed (site 2) and a third around the base of a dead tree (site 3) where no fruitbodies of mycorrhizal fungi had been recorded. At each site up to thirty planting positions were chosen. Half of these were first cored (20 cm deep and 20 cm diameter) and the core replaced *in situ* - inoculum within the cores had thus been severed from any contact with the mature tree or surrounding soil (FLEMING, 1984); the other half were left undisturbed. A non-mycorrhizal birch seedling was planted into each position on 31 May 1984; the seedlings were then 81 days old with a mean shoot height of 24 ± 1.5 (s.e.) mm and oven-dry weight of 10.5 ± 1.59 mg, and they bore 55 ± 5.0 uninfected root tips. The seedlings were left unattended for one growing season and those surviving were harvested on 14 March 1985 and assessed for numbers and types of mycorrhizas.

RESULTS AND DISCUSSION

Glasshouse bio-assay

Because the results obtained in 1982 and 1984 were similar, they have been combined. When soil samples were sieved (Table 1), mycorrhizas of *A. muscaria*, *L. roseofracta*, *Tricholoma fulvum* (D.C.:Fr.) Sacc., *Lactarius tabidus* Fr. and *Hebeloma* sp. were found beneath most or all of the respective fruitbodies and were often the dominant mycorrhizal types in these soil samples. A distinct mycorrhizal type was found only beneath the fruitbodies of *Cortinarius* sp.; these mycorrhizas are similar to some reported descriptions of *Cortinarius*-type mycorrhizas (eg. GODBOUT & FORTIN, 1985) and in this study are assumed to be attributable to *Cortinarius*. In contrast to the findings above, mycorrhizas of *Laccaria laccata* were found in few soil samples beneath *Laccaria* fruitbodies and were never the predominant type in these samples. These results closely resemble those of DEACON *et al.*, (1983) for fruitbodies collected at Bush Estate.

When seedlings were grown in soil samples in a glasshouse, *Amanita* and *Leccinum* did not form mycorrhizas in soils from beneath their fruitbodies. *Lactarius* and *Hebeloma* formed mycorrhizas on many seedlings in their respective soils whilst *Laccaria, Cortinarius* and *Tricholoma* formed mycorrhizas on fewer seedlings (Table 1). The results for *Laccaria* can perhaps be explained by the low inoculum densities of this fungus in the soil samples (Table 1) but the results for *Tricholoma* and *Cortinarius* cannot be explained in this way. So it seems that three types of behaviour can be distinguished, represented by (1) *Amanita* and *Leccinum,* (2) *Hebeloma* and *Lactarius*, and perhaps *Laccaria*, and (3) *Cortinarius* and *Tricholoma.* Behaviour of type (1) above corresponds to the behaviour of 'late-stage' fungi as defined by DEACON *et al.*, (1983). Behaviour of type (2) corresponds to that of 'early-stage' fungi, though the inclusion of *Lactarius tabidus* in this group is interesting because DEACON *et al.*, (1983) found *Lactarius pubescens* to behave like a 'late-stage' fungus. Behaviour of type (3) seems to be intermediate between the other groups and does not correspond to any previously recognized pattern.

All of 20 naturally regenerating seedlings collected from the woodland bore a predominant mycorrhizal type identified as *Elaphomyces granulatus* Fr.; several fruitbodies of this fungus were present below-ground and they were encrusted with this mycorrhizal type (MILLER & MILLER, 1984). Also, all 16 seedlings grown in a glasshouse in soil from this site bore large numbers of *Elaphomyces*-type mycorrhizas. On this basis *E. granulatus* seems to correspond to the early-stage category of fungi (type 2 above).

The final column of Table 1 shows the range of mycorrhizal types other than 'fruitbody types' that occurred in soils from beneath fruitbodies or on seedlings grown in these soils. It is notable that virtually no other mycorrhizal types occurred in soil samples from beneath *L. roseofracta* and *T. fulvum*. More mycorrhizal types occurred on seedlings than were observed within soil samples, fewest types occurring on seedlings grown in soils with the highest density of inoculum of a single fungus, such as *Leccinum, Tricholoma* and *Hebeloma*. Of further interest, *Leccinum* or *Amanita* mycorrhizas were found on 12 of the 99 seedlings grown in soil collected from beneath fruitbodies other than *A. muscaria* or *L. roseofracta* (and were dominant on 7 seedlings), even though these fungi did not form mycorrhizas on seedlings grown in soils from beneath their fruitbodies. This occurence warrants further study; it perhaps indicates depletion of nutrient reserves after fruiting (WESSELS & SIETSMA, 1979) and might be relevant to the spatial changes of mycorrhizas or their fruitbodies that are observed as trees age (FLEMING *et al.*, 1984).

Field outplanting

Many of the outplanted seedlings died, probably because of drought, leaving only 6 - 9 replicates of each treatment for sampling (Table 2). Even from these few remaining seedlings it is clear that *Amanita, Leccinum* and *Lactarius* could colonize seedling root systems from naturally occurring inocula; *Lactarius* and *Leccinum*, at least, could do so even in cored positions, when their inocula had been separated from a food base provided by nearby mature trees. Only *Amanita* failed to colonize

Table 1. Occurrence of mycorrhizal types : (A) in soil collected from beneath fruitbodies of seven mycorrhizal fungi in Struan Wood, and (B) on seedlings grown in the same soils in a glasshouse.

Fruitbody type	Soil samples or seedlings		Percent root tips with fruitbody type$^{\Delta}$	No. of other mycorrhizal types*
	No. examined	No. with fruitbody type (dominant in parentheses)$^{+}$		
A (Soils)				
Amanita muscaria	8	6(5)	nd	4 (*Leccinum*)
Leccinum roseofracta	3	3(3)	nd	0
Tricholoma fulvum	4	4(4)	nd	1 (*Leccinum*)
Cortinarius sp.	11	7(5)	nd	8 (*Lactarius, Amanita, Leccinum, Laccaria*)
Laccaria laccata	11	4(0)	nd	10 (*Leccinum, Lactarius, Amanita, Hebeloma*)
Lactarius tabidus	8	8(4)	nd	4 (*Leccinum, Hebeloma*)
Hebeloma sp.	6	6(5)	nd	2 (*Tricholoma*)
B (Seedlings)				
Amanita muscaria	19	0(0)	0	7 (*Leccinum, Lactarius, Laccaria*)
Leccinum roseofracta	3	0(0)	0	6 (*Lactarius, Cortinarius*)
Tricholoma fulvum	17	9(3)	12	3
Cortinarius sp.	17	7(5)	17	12 (*Amanita, Leccinum, Lactarius, Laccaria*)
Laccaria laccata	30	17(9)	37	12 (*Lactarius, Leccinum, Hebeloma*)
Lactarius tabidus	20	18(12)	44	9 (*Amanita, Leccinum*)
Hebeloma sp.	15	14(10)	44	6 (*Lactarius*)

* Only types referred to in the text are named.

$^{+}$ Fungi were regarded as dominant if they accounted for at least 50% of mycorrhizal tips.

$^{\Delta}$ nd, not determined.

Table 2. Occurrence of predominant mycorrhizal types on birch seedlings grown at 3 sites within Struan Wood in isolated (cored) and non-isolated positions.

	Mycorrhizal type	No. seedlings colonized by each mycorrhizal type		Mean No. root tips of each mycorrhizal type (±s.e.)	
		Cored	Non-isolated	Cored	Non-isolated
Site 1*	*Amanita*	0/7	4/6	0	8±5.2
	Unknown type 11	2/7	3/6	22±16.1	14±9.3
	Total mycorrhizas	-	-	52±12.2	26±10.4
	Total roots	-	-	109±18.2	63±15.4
Site 2*	*Leccinum*	4/9	3/8	14±10.8	11±6.9
	Lactarius	3/9	2/8	3±2.6	1±0.7
	Total mycorrhizas	-	-	57±16.4	54±12.8
	Total roots	-	-	85±16.0	88±13.8
Site 3*	*Lactarius*	5/9	4/9	17±6.0	12±6.8
	Cenococcum	6/9	4/9	12±4.4	13±7.7
	Total mycorrhizas	-	-	89±13.7	62±10.5
	Total roots	-	-	120±12.8	95±9.3

*Site 1, near *Amanita muscaria* fruitbodies; site 2, near *Leccinum roseofracta* and *Laccaria laccata* fruitbodies; site 3, base of a dead tree.

seedlings grown in cored positions. Table 2 lists only some of the mycorrhizal types found on the seedlings, because no type occurred in significantly different numbers on seedlings in isolated (cored) compared with non-isolated planting positions. Of interest, the numbers of mycorrhizas on seedlings were as great around the base of a dead tree (site 3) as around living mature trees (Table 2); even *Lactarius*, which was thought from previous work to require a food base for infection (FLEMING, 1984), colonized seedlings around the dead tree.

Current status of the 'early-stage'/'late-stage' distinction in mycorrhizal fungi.

Much evidence from observation and experiment suggests major behaviourial differences among mycorrhizal fungi, for which the terms 'early-stage' and 'late-stage' were suggested (DEACON *et al.*, 1983). In contrast to late-stage types, early-stage were seen to fruit earlier on an experimental birch plot on the Bush Estate (MASON *et al.*, 1982), their mycorrhizas predominated near the periphery of the root zone of trees (FLEMING *et al.*, 1984), they formed mycorrhizas readily on seedling roots in glasshouse conditions from naturally occurring or artificially introduced inocula (DEACON *et al.*, 1983) and their spores were shown readily to establish mycorrhizas on seedlings (FOX, 1983). Some of the work in this paper supports this distinction insofar as *Amanita* and *Leccinum* (late-stage) formed few mycorrhizas on glasshouse-grown seedlings from naturally-occurring inocula in soil (Table 1) whereas *Hebeloma* (early-stage) formed many mycorrhizas in equivalent conditions. Intermediate behaviour seemed to be shown by *Cortinarius* and *Tricholoma*, two genera which have so far received little attention in this respect. Nevertheless, some of our results contrast with those from Bush Estate. In particular, *L. tabidus* colonized many seedlings in the glasshouse, and *Lactarius*-type mycorrhizas occurred frequently on seedlings in the field, regardless of whether they were grown in isolated or non-isolated positions; this behaviour differs from that of *L. pubescens* and *L. glyciosmus* (Fr.:Fr.) Fr. on the Bush Estate (FLEMING, 1984). *Leccinum roseofracta* also behaved differently in Struan wood compared with on the Bush Estate (FLEMING, 1984) insofar as it colonized a few seedlings in soil in the glasshouse (Table 1) and in cored planting positions in the field. It seems, therefore, that soil or other site factors can modify the behaviour of a mycorrhizal fungus and that the concept of early-versus late-stage must be viewed in relation to soil and other biotic and abiotic variables. In this respect it may be significant that, unlike the soil at Bush Estate, there was frequently a surface accumulation of humus and litter at Struan wood. Such organic enrichment of the soil surface, typical of mature woodlands, apparently coincides with changes in the types of fruitbody of mycorrhizal fungi that are recorded between pioneer and mature birch woodland (WATLING, 1984); whether or not there is a causal relationship between these changes remains to be investigated.

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